

## NOTES AND COMMENTS



# Honey bee sacbrood virus infects adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae)

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The small hive beetle (SHB), *Aethina tumida*, is a parasite and scavenger of honey bee, *Apis mellifera*, colonies and has become an invasive species (Neumann and Ellis, 2008). Free-flying adult SHB invade host colonies over a distance of several km (Neumann and Elzen, 2004), thereby offering a potential means for transmission of honey bee pathogens, independently of bees and beekeepers. While recent data suggest that SHB may be a biological vector of deformed wing virus (DWV; Eyer *et al.*, 2009), no other honey bee virus infections have yet been reported. Here, we present results of laboratory experiments suggesting the food borne infection of adult SHB by sacbrood virus (SBV).

A piece of comb [7 × 10 cm] with open and sealed brood from a honey bee colony infected with DWV was placed into a plastic box [14 × 19 cm; hole with mesh wire on top 8 × 13 cm]. While adult workers from this colony showed clinical symptoms of DWV, no clinical symptoms of SBV were observed in the brood (Chen and Siede, 2007). Twenty adult SHB (24-48 h old, unfed) from a virus free laboratory culture (Eyer *et al.*, 2009) were placed in the box, which was maintained for seven days in an incubator at 30°C and 60% R.H. After incubation, all SHB were removed and immediately stored at -80°C until RNA could be extracted and analyzed individually for the presence of virus RNA using established strand-specific RT-PCR methods (Yue and Genersch, 2005; Chen and Siede, 2007). Additionally, SHB produced from the laboratory culture were individually analyzed for virus infection as a negative control. To confirm the specificity of the PCR, fragments amplified from both positive and negative strands of viral RNA were purified using the Wizard PCR Prep DNA Purification System (Promega, Madison, WI, USA) and sequenced in both forward and reverse directions. The nucleotide sequences of PCR products were analyzed and compared with

sequences published at the GenBank, National Center for Biotechnology Information, NIH (<http://www.ncbi.nlm.nih.gov/>) using Blast.

Four out of 12 SHB tested were found to be infected with SBV, the amplified sequences showing 97% homology to SBV. The two sequenced samples shared 100% sequence homology. Therefore, only one sequence was submitted to GenBank (accession number: FJ347141). All of the tested SHB were also DWV positive (Eyer *et al.*, 2009). SHB obtained directly from the laboratory culture were negative for both SBV and DWV infection (N = 20). Using negative strand specific PCR (Yue and Genersch, 2005), we also found a 824-bp long band of the negative stranded RNA of SBV in two out of the four SHB that were fed on honey bee brood and that were previously identified as being SBV positive by RT-PCR assay by the presence of positive stranded RNA of this particular virus. No positive control was included in the assay to exclude the possible chance of contamination. The specificity of individual PCR bands was confirmed by sequencing.

Our data show for the first time that SHB can become naturally infected by SBV via feeding on bee brood. Moreover, we can confirm infections of single hosts by multiple honey bee viruses (Chen and Siede, 2007). The detection of minus stranded RNA of SBV suggests virus replication in SHB. These observations indicate that SHB may act as a biological vector for SBV in a similar manner to that already suggested for DWV (Eyer *et al.*, 2009). If so then this could indicate a novel and unexplored route for honey bee virus transmission.

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